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the most abundant and previous studies have indicated that cytokine release by adipose tissue macrophages depends on adiposity. However, the mechanism underlying this association is unknown. In the present study, we explored the possibility that adipocytes modulate the phenotype of macrophages, thereby contributing to obesity-induced changes in adipose tissue.
Methods: Adipocyte conditioned media (ACM) were generated by culturing adipocytes from IFP (OA patients) or subcutaneous adipose tissue (ScAT, healthy donors) for 24hrs. Protein and lipid fractions were isolated using TBME and resuspended in medium. Macrophages were obtained by differentiating purified $CD14^+$ monocytes of healthy individuals in the presence of GM-CSF for 7 days. ACM or different fractions were added for the last 48h, while LPS was added during the last 24hrs of culture. IL-12 and TNFα cytokine secretion was measured by ELISA. The T cell stimulatory capacity of ACM-conditioned macrophages was measured in a 4-day mixed lymphocyte reaction. T cell proliferation was measured by tritium-thymidine incorporation.
Results: Treatment of macrophages with ACM resulted in a strong reduction in IL-12 secretion upon LPS stimulation, whereas TNFα remained unaffected. In addition, ACM-conditioned macrophages had an increased T cell stimulatory capacity. These effects were observed with both IFP- and ScAT-derived ACM. Interestingly, the inhibition of IL-12 release correlated to the Body Mass Index (BMI) of the IFP adipocyte donor. Separation of protein and lipid fractions of ACM indicated that the IL-12 inhibition was mediated by the lipid fraction. Current research investigates the lipid(s) responsible for the observed effects.
Conclusions: Macrophage function is modulated by soluble factors secreted by adipocytes. These factors appear to reside in the lipid fraction of the ACM. Interestingly, the inhibition of IL-12 release correlates with BMI of the adipocytes donor, indicating that obesity-related changes in macrophage phenotype could be mediated by adipocytes.

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MAINTENANCE OF THE PRIMARY CILIUM BY IFT88 PLAYS A ROLE IN THE CHONDROCYTE INFLAMMATORY RESPONSE TO INTERLEUKIN-1.

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Purpose: To understand the role of the chondrocyte primary cilium in inflammatory signalling. The primary cilium is a relatively understudied organelle protruding outward into the extracellular space, decorated with receptors. Its core tubulin structure and 'proteome' is maintained by a specific set of proteins including dynein motors and those of the intra-flagellar transport (IFT) family. Many forms of arthritis exhibit elevated levels of the quintessentially pro-inflammatory cytokine IL-1, which up-regulates the associated pro-catabolic signalling pathways. Recent studies have shown that the primary cilium is involved in chondrocyte mechanotransduction, observed changes in cilia length in osteoarthritis and that hedgehog signalling, which takes place on the cilium is critical in the pathogenesis of OA. Here we tested the hypothesis that the primary cilium is involved in mediating the pathological response to inflammatory cytokines and that this occurs through cytokine-induced regulation of cilia structure.
Methods: To test this we exploited a hypomorphic mutation of Tg737, encoding for IFT88, which abolishes genesis and growth of the primary cilium. WT and Tg737 chondrocytes were cultured in monolayer and treated with IL-1 (10ngml-1). Prostaglandin E2 (PGE2) levels in media were assessed by ELISA and nitric oxide release indicated by the Greiss assay. The cilium was labelled with anti-acetylated alpha tubulin antibodies and imaged using confocal microscopy. 3D z-stack reconstructions were used to assess ciliation and lengths measured using ImageJ software.
Results: IL-1 exposure of WT chondrocytes to IL-1β resulted in dramatic elevations of PGE2 and nitric oxide levels in the culture media. Intriguingly for the mutated cells, which lacked cilia, IL-1-induced PGE2 release was significantly attenuated and IL-1-induced nitric oxide release abolished. To assess if IL-1 elicits its effects by direct influence on cilia structure we assessed cilia length in primary cultures where we observed a consistent ~50% increase in length with a range of IL-1 treatments including different concentrations, times and subtypes. Pharmacological inhibition of PKA, with the small peptide inhibitor PKI and H89, abolished this IL-1 induced increase, which we believe may be a result of complex interference with cAMP balance. Additional studies using pharmacological inhibitors also indicate roles in IL-1-induced cilia elongation for PKC and the MEK-ERK pathways, already known to be downstream of IL-1 effects.
Conclusions: In conclusion these data show fascinating preliminary evidence that the primary cilium may have a role in cytokine-induced inflammation. Due to the localised and specific nature of much of the cilia proteome, a mechanistic understanding of the role of IFT and cilia structure in inflammation may lead to novel therapeutic targets for arthritis and in other pathologies with an inflammatory component.

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DEGREE OF SYNOVITIS ON MRI IS CORRELATED WITH HISTOLOGICAL AND MACROSCOPIC FEATURES OF SYNOVIAL TISSUE INFLAMMATION IN KNEE OSTEOARTHRITIS.

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Purpose: Synovitis is often present on MRI of OA knees and is an important determinant of pain. To better understand the nature of synovitis seen on MRI we compared microscopic and macroscopic features of synovial tissue inflammation with synovitis grade on contrast enhanced (CE) MRI.
Methods: 22 patients (mean age 61±7 years, 73 % women, mean BMI 30±5 kg/mm^2) with symptomatic radiographic knee OA attending the rheumatology outpatient clinic were included.
Arthroscopy of the index knee was performed and macroscopic features (neovascularization, villi, fibrin and hyperplasia) were scored (0-4). Furthermore, 15-20 synovial biopsies per knee were obtained. After haematoxylin and eosin staining, synovial tissue samples were microscopically scored on features: synovial lining layer hyperplasia/enlargement, activation of resident cells/stroma and degree of inflammatory infiltrates. Each feature was scored from 0-3 and a total sum score per patient was devised. Mean total scores (0-9) by 3 observers were used.
Saggital and axial T1-weighted Contrast enhanced (CE) MRI images (3T) were used to semi quantitatively score synovitis at 11 different sites according to Guermazi et al, ranging from 0-22 [1]. Self reported pain was assessed by visual analogue scale (VAS, 0-100). Pearson correlations adjusted for age were used for correlation between total histology synovitis score and total MRI score. Spearman rho correlations were used for correlation between total histology score and macroscopic features. Both were calculated by SPSS 16.0.
Results: The mean (SD) synovitis score on MRI was 7.8 (3.9), representing a mild synovitis, and mean (SD) histology score was 2.1 (1.5). Median (range) score of macroscopic features (0-4) were 2.0 (1.0-4.0) for neovascularization, 1.0 (0.0-3.0) for hyperplasia, 2.0 (0.0-4.0) for villi and 2.0 (0.0-3.0) for fibrin. Synovitis score on MRI correlated significantly with microscopic synovitis score [$r = 0.5$, $P = 0.019$] and macroscopic neovascularization score [$r = 0.6$, P 0.002] and hyperplasia [$r = 0.4$, $P = 0.40$]. Furthermore statistically significant correlation between microscopic synovitis score and macroscopic neovascularization [$r = 0.5$, $P = 0.012$] existed. No significant correlations with VAS pain were seen.
Conclusion: Synovitis severity on T1 weighted CE MRI images is significantly correlated with both macroscopic and microscopic features of synovitis in patients with knee OA. No association with severity of pain was seen. Therefore CE MRI evaluation is a reliable, non invasive way to determine synovitis severity in OA patients.

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TOLL-LIKE RECEPTORS 1, 2, 4, 6, MYD88 OR NALP3 HAVE NO EFFECT IN A MURINE MODEL OF OSTEOARTHRITIS.

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Purpose: Innate immune response components such as Toll-like receptors (TLRs), NLRP3 inflammasome and the intracellular signaling molecule MyD88 are all expressed by synovial membrane and chondrocytes. Previous studies have shown that TLR and NLRP3 inflammasome act in concert to increase IL-1β secretion by activated synovial macrophages and that IL-1β could be an important mediator involved in the pathogenesis of osteoarthritis (OA). The aim of our study was to evaluate the role of various TLRs, NLRP3 and the signaling molecule MyD88 in a murine model of OA induced by knee menisectomy (MNX, surgical removal of the medial meniscus).
Methods: Knees from 8-10 weeks old C57Bl6 wild type female (n=7), knock-out TLR-1 (n=5), -2 (n=7), -4 (n=8) -6 (n=5), NLRP3 (n=9) or MyD88 (n=7) were menisectomized, using the sham-operated contralateral knee as a control. 8 weeks later mice were sacrificed and knee cartilage destruction evaluated by histology using the OARSI scoring method. In addition, apoptotic chondrocytes were quantified in MyD88- and NLRP3-deficient knees. Finally, synovial inflammation was also scored.
Results: Operated knees exhibit OA symptoms at 8 weeks post-surgery compared to sham-operated knees as evidenced by both femoral and tibial cartilage degradation (increased OARSI score parameters, increased chondrocyte apoptosis) and by synovial inflammation. In menisectomized TLR-1, -2, -4, and -6 deficient mice, severity and extent of cartilage lesions and synovial inflammation were similar to that in MNX wild-type mice. Using the same approach, we found that the phenotype of NLRP3-deficient mice was similar to that of wild-type mice. Accordingly, we did not find a significant effect of MyD88 deletion, as cartilage erosion and synovial inflammation were similar in MNX knock-out and wild-type mice.
Conclusions: Knee MNX recapitulates features of OA, i.e, cartilage destruction and synovial inflammation. Deficiency of TLRs, NLRP3 inflammasome and their signaling molecule MyD88 did not impact on the severity of experimental OA. Our results suggest that NLRP3 inflammasome is either not involved in IL-1β activation or that IL-1β is not a key mediator in this murine OA model. This latter hypothesis is strengthened by the lack of efficiency of IL-1β antagonists in the treatment of OA.

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CELECOXIB DECREASES THE PRODUCTION OF CYTOKINES BY OSTEOARTHRITIC CARTILAGE AND SYNOVIUM WITHOUT AFFECTING CARTILAGE MATRIX DEGRADATION.

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Purpose: Recently, we reported a coculture model with osteoarthritic cartilage and synovial tissue that is more representative for osteoarthritis (OA) and useful to study mechanisms of action of OA therapies. Celecoxib, a COX-2 inhibitor, is frequently used for the treatment of osteoarthritis. However, its effect on cartilage metabolism has been the subject of much debate. Moreover, it is unclear how celecoxib affects the levels of soluble mediators in the joint. In the current study we investigate the effect of celecoxib on cartilage matrix turnover and the secretion of soluble mediators by cartilage and synovium in a coculture of OA cartilage and synovial tissue.
Methods: OA cartilage and OA synovium explants were cultured alone or in coculture for 21 days with the addition of celecoxib at 0.1, 1.0 and 10 μM. To study cartilage matrix turnover glycosaminoglycan (GAG) content, production and release were determined. ELISAs for IL-1α, IL-1ß, IL-4, IL-6, IL-8, IL-10, IL-13, OSM, MCP1, NGF, leptin, adiponectin and PGE2 were performed on conditioned medium of day 4. For statistical analysis, univariate analysis of variance was performed, with a randomized block design to correct for inter-donor variability. P values < 0.05 were considered significantly different.
Results: Both osteoarthritic cartilage and synovial tissue produced PGE2 and celecoxib inhibited the production in a dose dependent manner ($P < 0.05$; Fig. 1a). Celecoxib, irrespective of the concentration used, did not show an effect on GAG content, release and production in the coculture (Fig. 1b). Celecoxib at a concentration of 10 μM decreased the production of IL-1α, IL-1ß, IL-6, IL-10, OSM and NGF ($P < 0.05$). No effect was seen on leptin, adiponectin, IL-8 and MCP1. IL-13 and IL-4 were not detected (Fig. 1c).

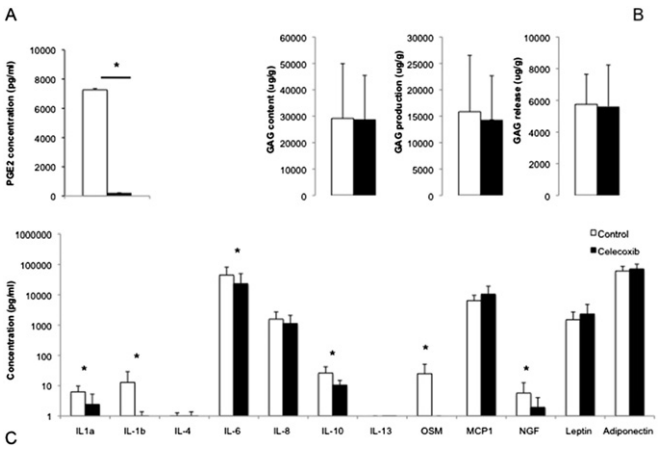

Figure 1. Effect of 10 μM celecoxib on PGE2 production, GAG content, production and release and soluble mediators in coculture of OA cartilage explants and synovial tissue. A: Celecoxib decreased PGE2 production (1 representative donor; * $P < 0.05$). B: No effect on GAG content, production and release (average +/- sd of 3 OA donors; n=4 donor/condition). C: Production of IL-1a, IL-1b, IL-6, IL-10, OSM and NGF is decreased by 10 uM celecoxib (average +/- sd of 3 donors; n=4 donor/condition; * $P < 0.05$).

Conclusion: This study shows that celecoxib decreased the production of multiple soluble mediators associated with inflammation and pain in OA. Although the production of multiple pro-inflammatory cytokines and PGE2 was decreased, no effect on cartilage matrix turnover was seen. The decreased production of mediators associated with pain in OA, such as NGF, IL-6 and PGE2 is in line with the effect on pain observed in patients treated with celecoxib.

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PRO-RESOLVING LIPID MEDIATORS ARE PRESENT IN THE JOINTS OF OSTEOARTHRITIS PATIENTS

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Purpose: Pro-resolving lipid mediators (LM), such as resolvins, protectins and maresins, are powerful molecules that have a critical role in limiting inflammation and promoting tissue regeneration. Biochemically they are derived from the enzymatic oxygenation of poly-unsaturated fatty acids by lipoxygenases (LOX). Some LOX involved in the generation of pro-resolving LM have been suggested to play a role in the pathophysiology of osteoarthritis (OA) in humans. However, it is currently unclear whether pro-resolving pathways are activated in the osteoarthritic joint, as the presence of pro-resolving LM or their precursors was not yet investigated. Therefore, we aimed at investigating the presence and abundance of pro-resolving LM and their precursors in synovial fluid (SF) of OA patients compared to Rheumatoid Arthritis (RA) patients.
Methods: SF samples from OA ($n = 8$) and RA ($n = 9$) patients visiting the outpatient clinic of the department of Rheumatology were collected. A targeted LC-MS(MS) lipidomics platform based on a QTrap mass spectrometer for the highly sensitive determination of several lipid mediators was used.
Results: The presence of LM could be proven in 4 of the OA and 5 of the RA patient samples. The pro-resolving LM resolvin D5 and maresin 1 were readily detectable and were accompanied by the presence of pro-inflammatory mediators such as leukotriene B4 and anti-inflammatory LM, such as lipoxin A4 and B4. Notably, the pro-inflammatory prostaglandins E2 and D2 could be detected in only 1 OA sample, while they were present in all 5 RA samples. Hydroxy derivatives of polyunsaturated fatty acids (HETE, HEPE and HDHA) which are precursors and biomarkers for LM were additionally detected in both patient groups.
Conclusions: This study indicates that a previously unrecognized pathway is likely activated in the knee joint of OA patients, as evidentiated by the presence of pro-resolving LM and their precursors in SF of these patients.